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# Low pH accelerates light-induced damage of photosystem II by enhancing the probability of the donor-side mechanism of photoinhibition

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**Abstract**

The effect of low pH on the process of photoinhibition was studied in isolated spinach thylakoids and PS II core complexes. Both the rate of photoinhibition of oxygen evolution and of D1 protein loss are substantially accelerated at pH 4.5 as compared to pH 7.0. Lowering the pH also affects the light-induced cleavage pattern of the D1 protein: at pH 6.0–7.0, the characteristic C-terminal fragments are of 8–10 kDa, whereas below pH 4.5, 23–24 kDa C-terminal products are accumulated. In addition, the predominant active oxygen species at pH 6.0–7.0 is singlet oxygen, but at low pH, it is replaced by hydroxyl radicals. Rapid D1 protein loss, which is accompanied by 23–24 kDa C-terminal fragments and hydroxyl radical production is characteristic of donor-side-induced photoinhibition. Thus, our results indicate that low pH conditions enhance light-induced damage to PS II function and protein structure by facilitating the donor-side mechanism of photoinhibition. The relevance of this effect to in vivo photoinhibition is discussed.

*Keywords:* Photosystem II; Photoinhibition; Low pH; D1 protein degradation; Oxygen evolution; Active oxygen species

## 1. Introduction

The primary target of photoinhibitory damage in oxygenic photosynthetic organisms is photosystem II (PS II) (for reviews, see [1–4]). A direct consequence of light-induced inactivation of PS II electron transport is the specific degradation of the D1 protein [5] and, to a lesser extent, of the D2 protein [6]. According to recent views, photoinhibition has two main mechanisms in isolated systems, which show characteristic differences regarding the site of electron transport lesion, the fragmentation pattern of the D1 protein and the production of active oxygen species.

Illumination of photosynthetic preparations with initially intact PS II electron transport leads to the so-called acceptor-side-induced photoinhibition. This mechanism is characterized by a block of electron transport at the PS II quinone electron acceptors leading to double reduction of $Q_A$ and to triplet formation at the reaction centre chlorophyll (Chl),

Abbreviations: Chl, chlorophyll; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DMPO, 5,5-dimethyl-1-pyrrolin *N*-oxide; Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*′-(2-ethanesulfonic acid); Mes, 2-(*N*-morpholino) ethanesulfonic acid; PS II, photosystem II; TEMP, 2,2,6,6-tetramethylpiperidine; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol

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$P_{680}$ [7–9]. The interaction of triplet Chl with molecular oxygen has been suggested to generate highly reactive singlet oxygen [8,10], which has been detected in photoinhibited PS II containing preparations [11–13]. The main products of D1 protein degradation are the 8–10 kDa C-terminal, and the corresponding 23 kDa N-terminal fragments in the acceptor-side-induced photoinhibition [14,15] indicating a preferential primary cleavage in the stromal loop between the fourth and fifth helices.

The so-called donor-side-induced photoinhibition is observed when the water-oxidizing activity of PS II preparations is already impaired before the illumination [16–19]. Under such conditions, PS II is extremely light sensitive, and the accumulation of highly oxidizing radical species, like $P_{680}^{+}$ and Tyr-$Z^{+}$, has been suggested to induce rapid damage to the function and protein structure of PS II [16–19]. Singlet oxygen is not observed in the donor-side-induced process [13]. In contrast, hydroxyl radicals are produced as the main active oxygen form [13]. During donor-side-induced photoinhibition, 23–24 kDa C-terminal and 8–10 kDa N-terminal fragments of the D1 protein are observed [15,20] corresponding to a preferential primary cleavage site in the lumenal loop between the first and second helices. A further 16 kDa C-terminal D1 fragment, indicating a cleavage site in the lumenal loop between the third and fourth helices, has also been detected under both acceptor- [15,21] and donor-side-induced photoinhibition [15,20,22]. However, this fragment is characteristic mainly of the donor-side conditions [22].

Recently, a further mechanism has been proposed to explain photoinhibition under very low light intensities [23]. According to this hypothesis, triplet $P_{680}$ can be formed via recombination of long-lived charge storage states of PS II, the higher S states on the donor-side and $Q_B^-$ on the acceptor-side, which then leads to damaging singlet oxygen formation as in the case of the acceptor-side mechanism of photoinhibition.

The importance of these well-characterized photoinhibitory mechanisms of isolated systems is not fully understood in vivo [24,25]. In most studies, the D1 protein was shown to be preferentially cleaved at sites exposed on the stromal side of the thylakoid lumen [26,27] showing the occurrence of the acceptor-side pathway. However, more recent studies indicated also concurrent acceptor- and donor-side type mechanism of D1 protein cleavage in vivo [28–30].

Low pH conditions are known to inhibit electron transport at the donor-side of PS II in isolated PS II preparations [31,32] (for review, see also [33]). This effect is likely to be related to the release of a $Ca^{2+}$ ion from the water-oxidizing complex [32,34]. Since various ways of inhibiting the donor-side activity of PS II, e.g. Tris-washing [16,17], hydroxyl amine treatment [18] or $Cl^-$-depletion [16,19], lead to increased light sensitivity, low pH may induce similar effect, but no evidence for this expectation has been provided yet. In intact cells, the pH of the thylakoid lumen is substantially decreased during illumination [35]. Acidification of the lumen down-regulates PS II which is believed to protect against photoinhibition either via enhanced energy dissipation through fluorescence quenching [36,37] and/or via the impairment of the oxygen-evolving complex [38]. On the other hand, the latter phenomenon is also suggested to induce photoinactivation of PS II in intact plants by enhancing the donor-side mechanism of photoinhibition [24,30,39].

In the present work, we investigated the effect of low pH on the characteristics of photoinhibition in isolated PS II preparations. Our results indicate that lowering the pH gradually enhances the donor-side pathway of photoinhibition at the expense of the acceptor-side mechanism.

## 2. Materials and methods

### *2.1. Sample preparation*

Thylakoid membranes were isolated from market spinach according to the method described in Ref. [40] and suspended in 50 mM Hepes (pH 7.0), 0.4 M sucrose, 15 mM NaCl and 5 mM $MgCl_2$. PS II core complexes were isolated from PS II membranes according to Ref. [41] and suspended in 50 mM Mes (pH 6.0), 0.4 M sucrose, 15 mM NaCl, 5 mM $CaCl_2$ and 2 mM dodecyl maltoside. Inactivation of the water-splitting complex was achieved by Tris-washing procedure in thylakoids [16] or by resuspension in 50 mM Tris (pH 8.0), 0.2 mM DBMIB (as artificial electron acceptor) and 2 mM dodecyl maltoside in the case of PS II core complexes [42].

### 2.2. Photoinhibitory treatment

Photoinhibitory treatment was performed at 22°C with 5000 $\mu$mol $m^{-2}$ $s^{-1}$ light provided by a KL-1500 lamp (DMP, Switzerland) through a glass optical fiber, in samples diluted to 100 $\mu$g Chl $ml^{-1}$ in the suspending buffers or, in the case of low pH studies, in 50 mM glycine (pH 4.5 or 4.0), 0.4 M sucrose, 15 mM NaCl and 5 mM $MgCl_2$.

### 2.3. Oxygen evolution

Oxygen evolution was measured with a Clark-type electrode (Hansatech, UK) in the corresponding buffers at the indicated pH, in samples containing 17 $\mu$g Chl $ml^{-1}$ and 0.3 mM dimethyl-*p*-benzoquinone as electron acceptor.

### 2.4. Active oxygen production

Active oxygen production was followed by spin trapping EPR spectroscopy. Singlet oxygen ($^1O_2$) was identified by measuring the EPR absorption of the stable nitroxide radical (TEMPO) produced in the reaction between $^1O_2$ and 10 mM TEMP during photoinhibition as described earlier [12]. Hydroxyl radical production was determined by EPR detection of the DMPO-OH adduct in the presence of 75 mM DMPO as spin trap according to Ref. [13].

### 2.5. Light-induced loss and fragmentation of the D1 protein

Light-induced loss and fragmentation of the D1 protein were studied using the immunoblotting technique as previously described [20]. The PS II polypeptides were resolved by denaturing electrophoresis in 12–17% (w/v) acrylamide gradient gels containing 6 M urea, and electroblotted on nitrocellulose membranes (0.45 $\mu$m, Sartorius, Germany). The loss of the D1 protein and its C-terminal fragments were identified with two different types of polyclonal antibodies: anti-D1, raised against the whole spinach D1 protein (kindly provided by Dr. R. Barbato) and anti-D1**C**, raised against a synthetic peptide corresponding to the C-terminus of pea D1 protein (a generous gift from Dr. P. Nixon), respectively. The immunoreaction was visualized with a biotinilated second antibody followed by extravidine-alkaline phosphatase conjugate and the appropriate chromogenic substrates. Densitometric analyses of the anti-D1 immunodecorated blots were performed using an LKB Laser Densitometer (Bromma, Sweden).

## 3. Results

### 3.1. Low pH accelerates photoinhibition of oxygen evolution and D1 protein loss

Photoinhibition of isolated spinach thylakoids was performed at two different pH values (7.0 and 4.5) and the time courses for inactivation of oxygen evolution were studied. In samples which were incubated in the dark at pH 4.5, only 60% of the PS II centres were active in oxygen evolution as compared to those at pH 7.0. As it is shown in Fig. 1, oxygen evolution is lost faster in samples photoinhibited at pH 4.5 ($t_{1/2} \approx 8–10$ min) than in the ones exposed to the same light intensity at pH 7.0 ($t_{1/2} \approx 30$ min).

In parallel with the inhibition of oxygen evolution, the time course for light-induced loss of the D1 protein was studied by immunoblotting with anti-D1 antibody (Fig. 2A) followed by densitometric analyses (Fig. 2B). At pH 7.0, D1 protein loss is character-

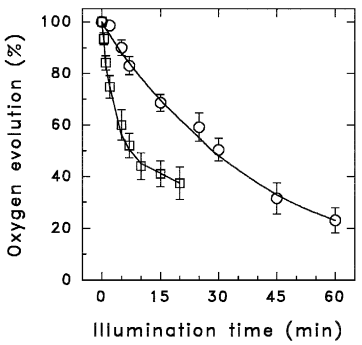


Fig. 1. Time-courses for inactivation of oxygen evolution during photoinhibition of thylakoids. Samples were illuminated at pH 7.0 (○) or 4.5 (□) followed by measurement of the oxygen evolution rates. The data expressed as percentages of the values obtained in the non-illuminated controls at the respective pH, are plotted as a function of illumination time, and represent averages from three independent experiments with the indicated standard deviations. The 100% oxygen evolution rates in the control thylakoids, suspended in the corresponding buffers at pH 7.0 and 4.5 were 210 and 125 $\mu$M $O_2$/mg Chl/h, respectively.

ized by $t_{1/2} \approx 100$ min, and accelerated to $t_{1/2} \approx 45$ min at pH 4.5 (Fig. 2B). Lowering the pH to 4.0, where oxygen evolution is almost completely inhibited even in the dark, further accelerates the loss of the D1 protein in high light ($t_{1/2} \approx 30$ min) (Fig. 2B). For comparison, the light sensitivity of the D1 protein was also studied at pH 7.0 in thylakoids whose oxygen-evolving activity had been inactivated by Tris-pre-treatment. Under these conditions, the time course for D1 protein loss is characterized by $t_{1/2} \approx 15$ min (Fig. 2B). These results show that low pH accelerates both the light-induced impairment of PS II electron transport and the loss of the D1 protein.

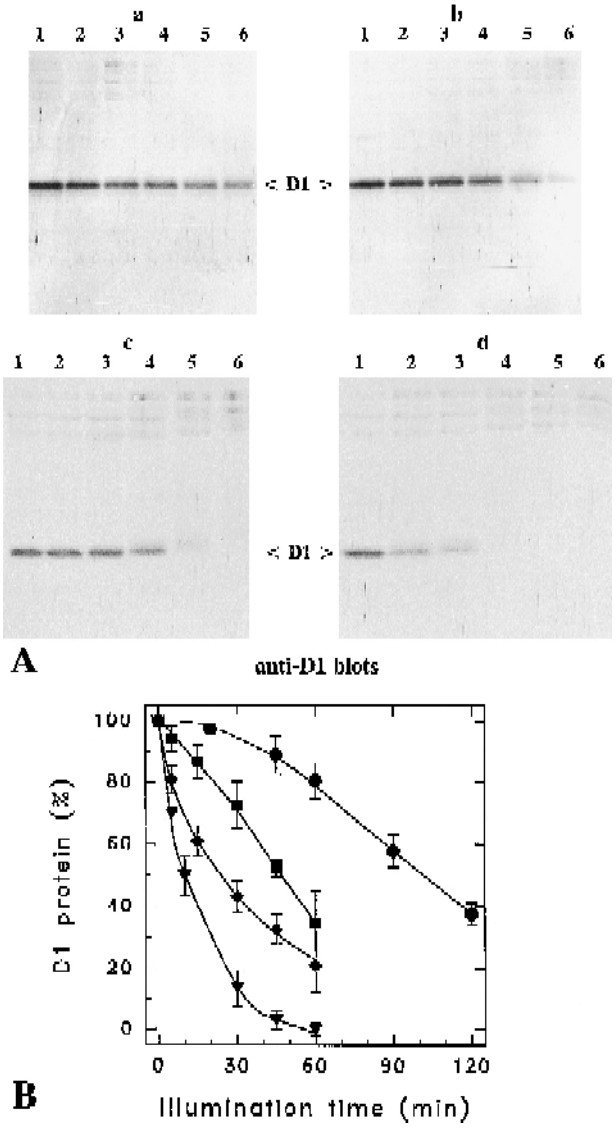


Fig. 2. Light-induced loss of the D1 protein at various pH values. *Panel A*: Immunoblots of photoinhibited thylakoids. Samples were illuminated for various periods of time at: (a) pH 7.0 (0, 20, 45, 60, 90 and 120 minutes; lanes 1 to 6, respectively), (b) pH 4.5 (0, 5, 15, 30, 45 and 60 minutes; lanes 1 to 6, respectively), (c) pH 4.0 (0, 5, 15, 30, 45 and 60 minutes; lanes 1 to 6, respectively) and (d) at pH 7.0 after inactivating the donor-side of PS II by Tris-washing (0, 5, 10, 30, 45 and 60 minutes; lanes 1 to 6, respectively). The blots were immunodecorated with an antibody raised against the whole D1 protein, under conditions where the staining response is linearly dependent on the amount of the D1 protein (i.e. 3 $\mu$g Chl per gel lane). *Panel B*: The time course for D1 protein loss. The amount of the D1 protein was obtained by densitometry of the immunodecorated blots (●, pH 7.0; ■, pH 4.5; ♦, pH 4.0 and ▼, pH 7.0 after Tris-washing) and plotted as a function of illumination time. The data are shown as a percentage of the values obtained in the non-illuminated controls at the respective pH and represent averages from three independent experiments with the indicated S.D.

### *3.2. Low pH induces donor-side cleavage of the D1 protein*

In order to obtain further information about the mechanism of photoinhibition at low pH, light-induced fragments of the D1 protein were immunodetected. In thylakoids exposed to photoinhibition at pH 7.0 for 2 h, no D1 fragmentation is observed using an antibody directed against the C-terminus of the D1 protein (Fig. 3, lanes 2–7). In contrast, a clear 16 kDa C-terminal fragment and traces of a 10 kDa band are accumulated during photoinhibition at pH 4.5 (Fig. 3, lanes 9–13). A similar induction of a 16 kDa D1 product has also been reported in photoinhibited thylakoids after Tris-inactivation of the water-oxidizing complex [22].

To detect small amount of fragments, the gels were overloaded, which resulted in a saturating immunostaining response for the main D1 band at 32 kDa. That is why the loss of the D1 protein is not obvious in these blots. Actually, at pH 4.5 the D1 band looks stronger after prolonged illumination times due to an enhanced diffusion of the band accompanied by an up-shift in the molecular mass. Besides the main D1 band and the lower molecular mass

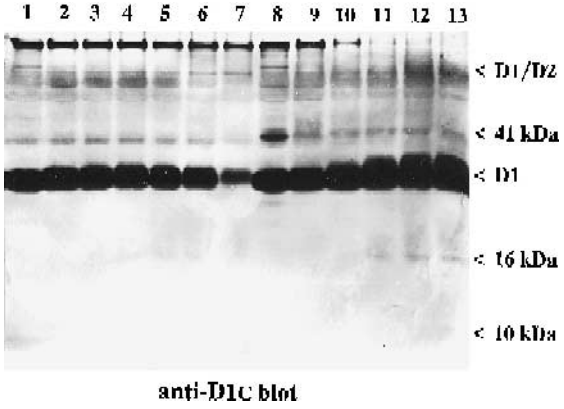


Fig. 3. Effect of low pH on the light-induced fragmentation of the D1 protein in thylakoids. Samples were photoinhibited for 15, 30, 45, 60, 90 and 120 min at pH 7.0 (lanes 2 to 7) or for 5, 15, 30, 45 and 60 min at pH 4.5 (lanes 9 to 13). Non-illuminated controls were kept in the dark for 120 min at pH 7.0 (lane 1) and for 60 min at pH 4.5 (lane 8). The blots were immunodecoraterd by an antibody raised against the C-terminus of the D1 protein, under conditions where the immunostaining response is saturating for the main D1 band at 32 kDa (i.e. 4 $\mu$g Chl per gel lane).

C-fragments, the D1/D2 heterodimer and a 41 kDa band also give immunostaining response. The latter product, which has been assigned to a cross-linked adduct of the D1 protein with the $\alpha$-subunit of cyt $b_{559}$ [46], is stronger in the dark-control thylakoids at pH 4.5 than at pH 7.0 (Fig. 3 lanes 1 and 8) and decreases during the course of photoinhibition.

Since D1 protein fragmentation is more easily detected in smaller fractions of PS II membranes than in thylakoids [14], the experiments were also performed using isolated oxygen-evolving PS II core particles (Fig. 4). At pH 6.0, the main fragments detected by the anti-D1C antibody are of 8–10 kDa and an additional band of 16 kDa (Fig. 4a). At pH 4.5, the 16 kDa fragment becomes the dominating C-terminal product and the intensity of the 8–10 kDa doublet decreases (Fig. 4b). By lowering the pH to 4.0, the 8–10 kDa fragments completely disappear, and in addition to the 16 kDa band, larger C-fragments of 23–24 kDa appear (Fig. 4c). When photoinhibition is performed under the conditions of fully inhibited donor-side activity, achieved by illuminating PS II core complexes at pH 8.0 in the presence of 0.2 mM DBMIB, the 23–24 and the 16 kDa C-fragments are clearly accumulated (Fig. 4d). These blots, similarly to those shown in Fig. 3, are overloaded in order to detect the fragments. As a consequence, D1 protein loss is not obvious in the main 32 kDa band. However, this band becomes more diffuse and shifted to higher molecular masses with the advance of photoinhibition. This behavior most likely indicates an oxidative damage to the undegraded D1 protein

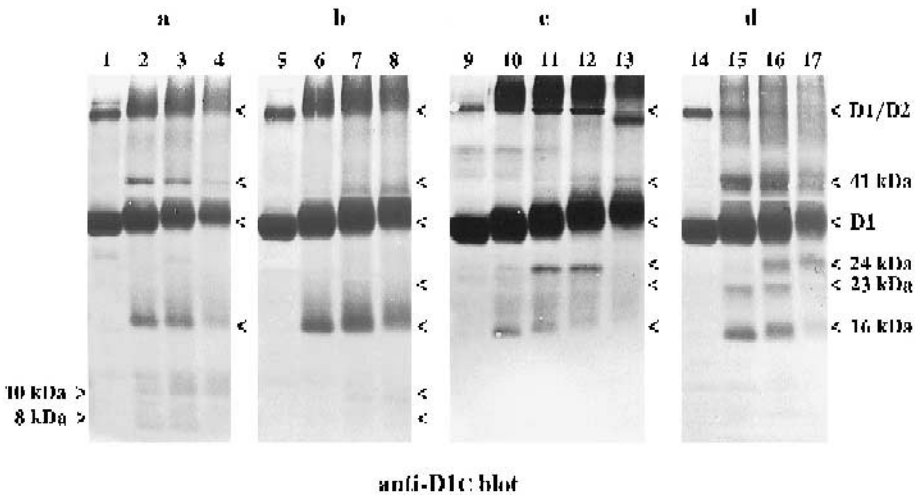


Fig. 4. Effect of low pH on the light-induced fragmentation of the D1 protein in PS II core complexes. Samples were photoinhibited at pH 6.0 (a), 4.5 (b), 4.0 (c) and at pH 8.0 in the presence of 0.2 mM DBMIB (d) for 30, 60 and 90 min (lanes 2–4, 6–8 and 15–17) or 15, 30, 60 and 90 min (lanes 10–13), respectively. Dark controls are shown in lanes 1, 5, 9 and 14. The immunodetection of the D1 protein and its C-terminal fragments was performed as described in Fig. 3.

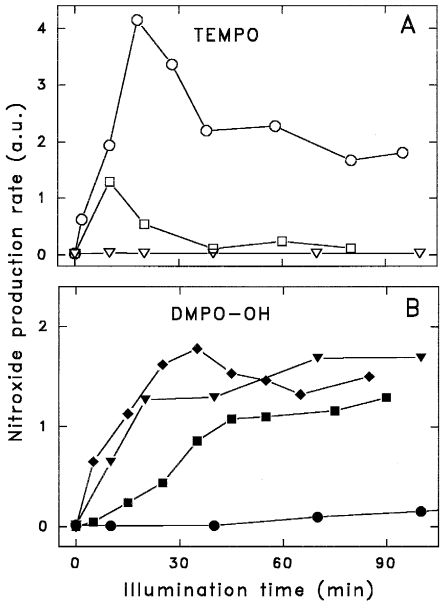


Fig. 5. Effect of low pH on the production of singlet oxygen and hydroxyl radicals during photoinhibition of thylakoids. Samples were photoinhibited at pH 7.0 (○, ●), 4.5 (□, ■) and 4.0 (◆) or at pH 7.0 after Tris-washing (▽, ▼). The production rates of TEMPO, arising from singlet oxygen trapping, (panel A) and of DMPO-OH, arising from hydroxyl radical trapping, (panel B) are plotted as a function of illumination time.

[15,47]. In addition to the fragmentation and oxidative modification of the D1 protein, high molecular mass aggregates are also induced, mainly at pH 4.0 (Fig. 4c) (see Section 4 below).

### *3.3. Light-induced formation of active oxygen species indicates enhanced donor-side pathway at low pH*

Photoinhibition of PS II has been shown to be accompanied by the formation of various active oxygen species [10,12,13]. Production of singlet oxygen, as detected by the formation of the EPR signal from TEMPO is observed during photoinhibition of intact thylakoids at pH 7.0, but largely suppressed at pH 4.5 (Fig. 5A). Inactivation of oxygen evolution by Tris-washing also diminishes singlet oxygen production at pH 7.0, in agreement with our earlier results [13]. Trapping of hydroxyl radicals by DMPO is negligible during photoinhibition of intact thylakoids at pH 7.0, but becomes dominant in Tris-washed samples (Fig. 5B and [13]). Photoinhibition at pH 4.5 considerably enhances the rate of DMPO-OH production, and at pH 4.0 similar rates of hydroxyl radical production are observed as in Tris-inactivated samples at pH 7.0 (Fig. 5B). Additional experiments carried out with chemically generated hydroxyl radicals demonstrated that their trapping by DMPO is not significantly different at pH 7.0 and 4.5 (not shown). Therefore, the observed differences should be interpreted as pH-related changes in hydroxyl production rates. However, it is of note that trapping of chemically generated $^1O_2$ by TEMP is diminished below pH 4.5 due to the protonation of the trap (not shown). Thus, this method was not used to monitor $^1O_2$ production during photoinhibition at pH 4.0.

## 4. Discussion

Low pH conditions are known to inhibit donor-side electron transport in isolated PS II preparations [31,32], most likely due to the release of a $Ca^{2+}$ ion from the water-oxidizing complex [32,34,38]. Since inhibiting PS II donor-side reactions by Tris-washing [17,18], hydroxyl amine treatment [19] or $Cl^-$-depletion [17,20] all induce donor-side type photoinhibition, the donor-side lesion of electron transport at low pH is also expected to facilitate rapid photoinhibition via the donor-side pathway. According to the results presented here, the damaging effects of photoinhibitory illumination are indeed accelerated at low pH both at the level of PS II activity and of the D1 protein damage. Acceleration of light-induced inhibition of oxygen evolution (Fig. 1) is an interesting observation because it shows that low pH enhances inactivation of electron transport in those centres which retain oxygen-evolving activity. A possible explanation for this effect is the decrease of binding affinity of $Ca^{2+}$ in the light-induced higher S states ($S_2$, $S_3$) relative to the dark stable lower ones ($S_0$, $S_1$) [34]. Thus, $Ca^{2+}$ release can be accelerated during the photoinhibitory treatment, which keeps about half of those centres that are still active in oxygen evolution in the higher S states. Addition of $Ca^{2+}$ to the thylakoid suspension increases the level of oxygen evolution retained after dark incubation at pH 4.5 by about 30%, however, the time course of photoin-

hibitory damage is not significantly affected (not shown). This effect most likely indicates that even a high concentration of $Ca^{2+}$ in the bulk medium (50 mM) can not compensate the decreased binding affinity in the higher S states at pH 4.5.

The rate of the D1 protein loss is accelerated to a similar extent as the inhibition of oxygen evolution by lowering the pH from 7.0 to 4.5 (Fig. 2B). In addition, at pH 4.0, where oxygen evolution is almost completely inhibited in the dark, the light-induced loss of the D1 protein proceeds with similar kinetics as observed at pH 7.0 in preparations in which the oxygen-evolving complex is inactivated by Tris-washing prior to the photoinhibitory illumination. These observations suggest that rapid loss of the D1 protein occurs in those PS II centres in which electron flow from the water-oxidizing complex had already been inactivated by low pH, i.e. the conditions for donor-side-induced photoinhibition are established.

The idea that increased light sensitivity of PS II at low pH is related to the impairment of donor-side electron transport is further supported by the characteristic pH-dependence observed in the fragmentation pattern of the D1 protein. At pH 6.0–7.0, which is optimal for the function of the water-oxidizing complex and the main pathway of photoinhibition is the acceptor-side type, the preferential cleavage site is located in the stromal loop between the fourth and fifth helices [14,24] (for review, see also [43]). This cleavage site is characterized by 8–10 kDa C-terminal and 23 kDa N-terminal fragments, among which we detected the C-products (Fig. 4a). At low pH (4.5–4.0) the 8–10 kDa C-terminal products gradually disappear, and are replaced by 23–24 and 16 kDa C-terminal fragments (Fig. 4b,c) in a very similar fashion to that observed at pH 8.0, under the conditions of donor-side-induced photoinhibition (Fig. 4d). These fragments indicate cleavage in the lumenal loops of the D1 protein between the first and second as well as between the third and fourth helices, respectively. Even though the 16 kDa C-terminal fragment is present at pH 6.0 in the isolated core particles and its intensity is higher than that of the 8–10 kDa C-fragments (Fig. 4a), it does not represent the preferential cleavage product at this pH [21,22]. This idea is supported by the complete lack of the 16 kDa D1 fragment in thylakoids at pH 7.0 and its induction at pH 4.5 (Fig. 3), similarly to that observed in Tris-treated thylakoids at pH 7.0 [22]. However, the presence of the 16 kDa C-fragment may indicate that in core particles photoinhibition proceeds via a mixture of the donor- and acceptor-side mechanisms even at pH 6.0. At this pH the dominating contribution comes from the acceptor-side pathway, but progressively replaced with the donor-side effects at lower pH values. This explanation is supported by the gradual change in the fragmentation pattern between pH 6.0 and 4.0 (Fig. 4a,b,c).

The pH-dependent formation of light-induced active oxygen species brings further evidence for enhanced donor-side type of photoinhibition below pH 4.5. At pH 7.0 the dominant active oxygen form is singlet oxygen, which is characteristic of acceptor-side-induced photoinhibition [12]. However, this species is gradually replaced with hydroxyl radicals, which dominate donor-side-induced photoinhibition [13], upon decreasing the pH to 4.0.

Our results show that in isolated systems low pH accelerates photoinhibition of PS II electron transport and D1 protein loss, concomitant with dominating D1 cleavage at lumenal loops, and hydroxyl radical production. These features are characteristic of donor-side-induced photoinhibition and show that low pH conditions enhance the probability of the donor-side mechanism relative to that of the acceptor-side one.

It is of note that the accelerated loss and fragmentation of D1 at low pH is accompanied by the formation of high molecular mass aggregates. This effect is most pronounced at pH 4.0 in the overloaded blots (Fig. 4c), but also present to some extent in the blots of Fig. 2A, where the staining response of the main D1 band is in the linear range. Thus, the rapid loss of the D1 protein at pH 4.0 may be partly due to aggregation. Similar aggregation effects have been observed during prolonged photoinhibition of isolated PS II core- and reaction centre complexes at neutral pH values [15,47] and assigned to singlet oxygen and hydroxyl radical attack on amino acid residues. Since the dominating active oxygen forms during photoinhibiton at low pH are hydroxyl radicals, these species could be responsible for the observed aggregation in our experiments. However, it is difficult to draw quantitative conclusions from the apparently larger amount of D1-containing aggregates relative to the C-terminal fragments concerning the contribution of

aggregation and fragmentation to the loss of D1 band. The amount of aggregates is negligible at pH 4.5 and not very large even at pH 4.0, under the conditions of linear immunostaining response in Fig. 2A. Thus, aggregation does not seem to be the major factor inducing D1 loss. The low amount of fragments could be caused by their rapid further degradation, which may not affect the aggregates to the same extent.

Based on our in vitro studies, light-induced acidification of the thylakoid lumen in intact cells appears to be a potentially dangerous situation for the function and integrity of PS II. This is in contrast to previous suggestions about the presumed protective role of low lumenal pH-induced inhibition of the water-oxidizing complex against both the donor- and acceptor-side photoinhibitory mechanisms [38]. Plants can avoid the hazardous situation imposed by the high-light-induced acidification of the thylakoid lumen, via enhanced excitation energy dissipation through the pH-dependent fluorescence quenching mechanisms, if it is poised so that efficient energy quenching comes in before the lumenal pH falls low enough to inhibit the donor-side reactions. The lumenal pH is expected to be around 5.5–5.0 during continuous illumination in vivo [44,45], which is already accompanied by strong fluorescence quenching. Since according to our results, donor-side-induced photoinhibition becomes dominant below pH 4.5, intact plants can probably avoid extensive donor-side-induced photoinhibitory damage. This is in agreement with reports showing the dominating cleavage site of the D1 protein located in the stromal loop between the fourth and fifth helices in vivo [26,27]. However, some enhancement of photoinhibition by the donor-side pathway upon acidification of the thylakoid lumen may occur already at around pH 5.0 and this effect may be responsible for the concurrent donor- and acceptor-side type cleavage of the D1 protein which is also observed in intact plants [28–30].

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